Microscopic esophagitis and Barrett’s esophagus: The histology report

Roberto Fiocca, Luca Mastracci, Massimo Milione, Paola Parente, Vincenzo Savarino

On behalf of the “Gruppo Italiano Patologi Apparato Digerente (GIPAD)” and of the “Società Italiana di Anatomia Patologica e Citopatologia Diagnostica”/International Academy of Pathology, Italian division (SIAPEC/IAP)

Abstract

Gastro-esophageal reflux disease (GERD) is the most common digestive disease in industrialized countries (Europe and North America) and is associated with microscopic changes in the squamous epithelium. However, biopsy is not presently included in the routine diagnostic flow chart of GERD. In contrast, esophageal biopsy is mandatory when diagnosing Barrett’s esophagus. High quality histology reports are necessary to provide information on diagnosis and can also be important for research and epidemiological studies. It has been evident for decades that pathology reports vary between institutions and even within a single institution. Standardization of reporting is the best way to ensure that information necessary for patient management is included in pathology reports. This paper details the histological criteria for diagnosing GERD-associated microscopic esophagitis, other forms of esophagitis with specific features and columnar metaplasia in the lower esophagus (Barrett’s esophagus). It provides a detailed description of appropriate sampling criteria, individual lesions and how they contribute to the histology report.

Keywords: Barrett’s esophagus; Esophagitis; Gastro-esophageal reflux disease; GIPAD report; Histopathology

1. Introduction

Gastro-esophageal reflux disease (GERD) is extremely widespread in industrialized countries (Europe and North America) with a prevalence of 10–20% [1]. The prevalence of Barrett’s esophagus among patients undergoing endoscopic examination is 1% and reaches 3% if only the patients with reflux symptoms are taken into account [2].

The modern approach to histological diagnosis of non-neoplastic diseases of the esophagus started with the advent of fiber optic endoscopy and the possibility of obtaining endoscopic biopsies. The first great contribution to a better understanding of microscopic lesions associated with gastro-esophageal reflux disease (GERD) came from Ismail-Beigi’s study [3], while the intricate story of Barrett’s esophagus (BE) began in 1950 [4].

The present document is an update to the “guidelines” issued by Gruppo Italiano Patologi dell’Apparato Digerente (GIPAD) in 1998 [5] and it covers the following topics:

1. Indications for esophageal biopsy
2. Microscopic esophagitis
   • Individual histologic lesions
• Histologic pattern of microscopic esophagitis
• Histologic lesions associated with other esophagitis (eosinophilic, infectious, non-infectious)

3. Barrett’s esophagus
• Definition
• Endoscopic description and biopsy sampling
• Individual histologic lesions

The asterisks in the present article express the levels of evidence, where one asterisk represents level 1 (evidence provided by single studies); two asterisks represent level 2 (evidence provided by some clinico-pathological studies, albeit with some notable discrepancies); and three asterisks represent level 3 (evidence consistently established by a large body of clinico-pathological and/or follow-up studies), as described more in detail in the Foreword in this supplement [6].

2. Clinical indication for esophageal biopsy

2.1. Gastro-esophageal reflux disease

There are no indications for routine esophageal biopsies in patients with esophageal or extra-esophageal symptoms of gastro-esophageal reflux disease [7]. The rationale underlying this statement is that histological findings do not seem to provide any additional information for patient management when compared with endoscopy. In other words, it is sufficient to distinguish erosive lesions of any degree by endoscopy (ERD) from non erosive form (NERD) in the absence of endoscopically recognizable lesions. Squamous epithelium histology can provide a better understanding of reflux disease at the mucosal level** [8] and enable comparative assessment of the effect of anti-reflux treatments** [9]. Biopsy contribution is significant for research purposes only, and presently it is not included in the routine diagnostic flow chart for GERD.

2.2. Barrett’s esophagus

Esophageal biopsy is crucial when diagnosing Barrett’s esophagus*** and for monitoring purposes after diagnosis. Columnar metaplasia [10] of the esophageal mucosa has to be histologically proven in order to correctly define this complication of GERD***.

2.3. Endoscopically suspected esophageal lesions

Esophageal biopsy is mandatory in all suspected lesions that are not clearly defined during endoscopy. Histological examination is required whenever an endoscopic finding at any level along the esophagus raises doubts concerning the nature of the lesion (benign vs. malignant) [11].

2.4. Eosinophilic esophagitis

Histology is mandatory in this condition. High eosinophil count (≥15/HPF) is required for the diagnosis of eosinophilic esophagitis in patients with dysphagia and atopy*** [12]. Biopsy specimens must be taken also from the proximal or middle esophagus.

2.5. Infectious esophagitis

Biopsy is required in order to differentiate with certainty viral or bacterial esophagitis from other types of esophagitis, although this is a relatively rare occurrence in clinical practice.

2.6. Clinical information

As in other digestive diseases, the following information should be provided when requiring a histological examination:
• reason for the examination;
• concomitant diseases (e.g. connective tissue diseases, skin diseases, allergies, etc.);
• endoscopic pattern;
• previous and current treatments;
• biopsy site;
• previous histological examinations.

A list of all useful clinical and endoscopic data would be too long and beyond the scope of this paper. The importance of endoscopic-biopistic correlations in the diagnosis of Barrett’s esophagus is covered in a subsequent section of this paper.

2.7. Normal esophageal mucosa (Table 1)

The esophageal mucosa is lined by stratified squamous epithelium. Sparse T lymphocytes (usually <10/HPF) in the suprabasal area, sparse Langerhans cells and very few mast cells [13] are constitutively present in normal esophageal epithelium. In contrast, no eosinophils or neutrophils are found. Esophageal biopsy specimens should include the full epithelial thickness, allow the recognition of the base of papillae and be well oriented.

3. Microscopic esophagitis

3.1. Definition

The term “microscopic esophagitis” refers to a group of histologic lesions observed in most patients with GERD, both erosive (ERD) and non-erosive (NERD). It is not to be confused with “esophagitis”, a term that in the gastroenterological literature designates endoscopically detectable erosive lesions.

Table 1
Criteria for normal esophageal mucosa***

| Basal layer thickness: <20% (at the Z line); <15% (in more proximal biopsies)† |
| Papillary length: <66% (at the Z line); <50% (in more proximal biopsies)† |
| Intraepithelial T lymphocytes: <10/HPF |
| Absence of intercellular spaces dilation |
| Absence of intraepithelial eosinophils and neutrophils |

†Figures refer to the full thickness of the epithelium.
3.2. Individual histologic lesions

None of the histologic lesions characterizing microscopic esophagitis is specific of and exclusive to reflux disease; the same lesions can be observed also in other types of esophagitis (infectious, eosinophilic, due to achalasia, etc.).

The assessment of histologic lesions should be performed in well oriented areas of biopsy specimens where lesions are most severe.

**Basal cell hyperplasia** – thickness of basal layer ≥20% (esophageal side of Z line)* [14] of total epithelial thickness; ≥15% (biopsies proximal to Z line)*** [3]. The upper limit of the basal layer is defined as the level where the nuclei of epithelial cells are separated by a distance greater than their diameter [15] (Fig. 1). Basal cell hyperplasia can be graded as mild (<30%) or marked (≥30%).

**Papillae elongation** – length of papillae ≥66% (esophageal side of Z line)* of total epithelial thickness [3,14]; ≥50% (biopsies proximal to Z line)*** [16]. The upper limit of the papilla is defined as the upper limit of the vessel running along its axis [15] (Fig. 2). It can be graded as mild (<75%) or marked (≥75%).

**Dilated intercellular spaces (DIS)** – irregular dilatations of intercellular spaces, detectable at light microscopy as optically empty bubbles or ladders. They are more prevalent in the lower half of the epithelium and around the papillae [15,17]. They must not be confused with “stretching” artefacts resulting from bioptic sampling and with intracytoplasmic vacuoles. They can be graded as small or large (< or > diameter of a small lymphocyte) (Fig. 3). Occasional small dilatations may also be found in the squamous epithelium of control subjects.

**Intraepithelial eosinophils and neutrophils** [18,19]. The presence of intraepithelial eosinophils (Fig. 4) is the main distinguishing feature of eosinophilic esophagitis (EE). The majority (85%) of patients with eosinophilic infiltration, however, are affected by GERD [20]. Intraepithelial neutrophils are a rare finding (<5%) in patients with NERD [14]; their presence is usually associated with endoscopic changes (ERD, infectious and non-infectious esophagitis).

**Erosion/healed erosion** – Erosion is characterized by the presence of necrosis and/or granulation tissue and/or fibrin with neutrophils, while healed erosion is characterized by the presence of fibrosis/granulation tissue covered by thin epithelium with regenerative changes in the absence of necrosis [15] (Fig. 5).

**Intraepithelial T lymphocytes** – Counting intraepithelial T lymphocytes in routine specimens is difficult due to the presence of other mononuclear cells, and their increase contributes little to the diagnosis of microscopic esophagitis [3,21]
Fig. 4. Marked infiltration of eosinophils in the squamous epithelium with a microabscess close to the surface. (H-E, 40×)

Fig. 5. Healed erosion is characterized by the presence of fibrosis and granulation tissue covered by thin epithelium with regenerative changes in the absence of necrosis. (H-E, 20×)

Other lesions – Capillary ectasia, ballooning and eosinophilic densification in the squamous epithelium have been described, but they contribute little to the diagnosis of microscopic esophagitis.

Basal cell hyperplasia and DIS are the individual lesions most contributing to a diagnosis of microscopic esophagitis, followed by papillae elongation and intraepithelial eosinophils (Table 2) [14,22]. Basal cell hyperplasia, papillae elongation, DIS and eosinophils show variable sensitivity and specificity. As none of these lesions is diagnostic of microscopic esophagitis, more than one individual histologic lesion is required for the diagnosis**. Moreover, one mild histologic lesion may be found also in biopsies of control subjects [14].

The sensitivity of histologic lesions such as intraepithelial neutrophils, erosion and healed erosion is very low***. They are found almost exclusively in subjects with ERD [14,23,24] and represent the most severe end of the spectrum; whenever they are found, microscopic esophagitis can be diagnosed regardless of the presence of other lesions**.

3.3. Limitations of histologic diagnosis

Histologic lesions in microscopic esophagitis may be focal and irregularly distributed [3,14,23,24]. Consequently, the assessment should be made in the most affected (and technically suitable) area.

The greater the number of biopsies, the higher the diagnostic sensitivity [23].

The most distal biopsies, especially those close to the Z line, are the most informative [14,23]; however, increased informativeness is accompanied by decreased specificity, i.e. one mild lesion may also be found in control subjects [14].

The assessment of basal cell hyperplasia and papillae elongation requires well oriented biopsies [14,25].

The sensitivity and specificity of individual lesions is variable among different studies and the results can either be disappointing or extremely promising [22,23]. Such variability is mainly due to case and control selection criteria [8].

Interobserver reproducibility of histologic lesions also varies: it is influenced by common training [14,15] and consequently it is lower in multicenter studies (Table 2). Discrepancies arise from the choice of the microscopic field to be assessed [15] and are minimized by the use of preselected photographic fields.

A scoring system including multiple histologic lesions could help to increase both sensitivity and specificity of histological findings [14,26].

The severity of GERD-related histologic lesions is strongly influenced by treatment with anti-secretory drugs and by anti-reflux surgery [15], hence the importance of knowing patient treatment history.

3.4. Biopsy sampling

As histologic lesions in GERD are usually limited to the distal esophagus, standard sampling should include the

<table>
<thead>
<tr>
<th>Individual histologic lesion</th>
<th>Sensitivity†</th>
<th>Specificity†</th>
<th>Reproducibility (k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cell hyperplasia</td>
<td>93%</td>
<td>45%</td>
<td>0.86†</td>
</tr>
<tr>
<td>Papillae elongation</td>
<td>62%</td>
<td>80%</td>
<td>0.87†</td>
</tr>
<tr>
<td>DIS</td>
<td>86%</td>
<td>70%</td>
<td>0.91†</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>49%</td>
<td>90%</td>
<td>0.87*</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>7%</td>
<td>100%</td>
<td>0.84*</td>
</tr>
<tr>
<td>Erosion</td>
<td>8%</td>
<td>100%</td>
<td>0.90*</td>
</tr>
<tr>
<td>Healed erosion</td>
<td>ND</td>
<td>ND</td>
<td>0.73*</td>
</tr>
</tbody>
</table>

last 2 cm above the Z line (2 biopsies at 2 cm and 2 biopsies on the esophageal side of the Z line)**. More proximal biopsies are less informative [14]. In eosinophilic esophagitis specimens should be taken from the proximal-middle esophagus too. Moreover, samples must be obtained from every endoscopically suspicious lesion.

3.5. Diagnostic categories

1. **Microscopic esophagitis:** this diagnosis is substantiated by the presence of more than one mild histologic lesion or erosion/healed erosion/intraepithelial neutrophils in GERD patients.

2. **Eosinophilic esophagitis** is a clinico-pathological condition characterized by [12]:
   - esophageal and/or upper gastrointestinal symptoms (dysphagia, food impaction, GERD-like symptoms, etc);
   - frequent association with a history of bronchial asthma;
   - normal pH values;
   - absent/poor response to high-dose proton pump inhibitors.

   In this clinical context, the diagnosis must be confirmed by the presence of $\geq 15$ intraepithelial eosinophils/HPF, especially forming microabscesses in the superficial layers of the epithelium*** (Fig. 4). Contrary to GERD-related microscopic esophagitis, the lesions are usually found also in the proximal part of the esophagus. High intraepithelial eosinophil counts are not exclusive to eosinophilic esophagitis, as they can be found also in GERD patients [20] and other clinical conditions.

3. **Infectious esophagitis.** Infectious esophagitis is usually characterized by necrosis of the mucosa (erosion/ulcer) and acute inflammation. Ulcers are associated with the presence of granulation tissue. These features are not specific and they can also be found in other types of esophagitis. Diagnosis must be based on the identification of viral inclusions and microorganisms by means of special staining, immunohistochemical or molecular tests. The most common lesions are described in Table 3.

4. **Non infectious esophagitis (Table 4).** Non infectious esophagitis (due to exposure to chemical or physical agents or drugs, or associated with diseases of other organs) are diagnosed mainly on the basis of clinical history, because as a rule no pathognomonic lesions are found. Esophageal involvement during bullous skin diseases is usually limited to the proximal part of the esophagus.

3.6. Special methods

Staining with hematoxylin and eosin is sufficient to diagnose microscopic esophagitis. For some specific types of esophagitis, histochemical staining (PAS/Grocot for fungal esophagitis) or immunohistochemical staining (CMV and HSV antibodies) may be useful. Identification of eosinophils may be hindered by Bouin’s fixation.

3.7. How to write the diagnosis

**Microscopic findings –** Number and site of esophageal mucosa specimens.

- Orientation: adequate/ not adequate.
- Histologic lesions:
  - basal cell hyperplasia (mild – marked)
  - papillae elongation (mild – marked)
  - dilated intercellular spaces (small – large)
  - intraepithelial eosinophils (maximum number/HPF)
  - intraepithelial neutrophils
  - erosion/healed erosion
- Other specific lesions (fungal forms, cytopathic viral changes, etc.).

**Diagnosis**

- Normal findings (no lesion)/Findings are not sufficient to justify the diagnosis of microscopic esophagitis (presence of 1 mild histologic lesion).
- Findings are “diagnostic” for microscopic esophagitis ($>1$ mild histologic lesion or erosion/healed erosion/intraepithelial neutrophils).

### Table 3

**Infectious esophagitis (from Lapertosa et al. [5], with amendments)**

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Clinical information</th>
<th>Guide lesions</th>
<th>Aspecific lesions</th>
<th>Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes simplex</td>
<td>Immunocompetence, site, endoscopic picture (vesicles, small ulcers)</td>
<td>Intranuclear eosinophilic inclusions (Cowdry A); multinucleate syncytia of squamous cells</td>
<td>Erosion and/or ulcer, mixed inflammatory infiltrate</td>
<td>HE IHC</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Immunocompetence, site, endoscopic picture (ulcer)</td>
<td>Cyto- and nucleo-megaly with nuclear inclusions</td>
<td>Macosal ulcers, mixed inflammatory infiltrate</td>
<td>HE IHC</td>
</tr>
<tr>
<td>HPV</td>
<td>Association with squamous papilloma and/or squamous carcinoma</td>
<td>Koilocytosis</td>
<td></td>
<td>HE IHC</td>
</tr>
<tr>
<td>Candida</td>
<td>Immuno competence, site (often multiple sites), endoscopic picture (erosions, ulcers, pseudomembranes)</td>
<td>Hyphae and spores</td>
<td>Erosion and/or ulcer, acute inflammation, cell debris, fibrin</td>
<td>HE PAS/Grocot</td>
</tr>
</tbody>
</table>

HE: hematoxylin-eosin; IHC: immunohistochemistry.
Table 4
Non-infectious esophagitis (from Lapertosa et al. [5], with amendments)

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Clinical information</th>
<th>Guide lesions</th>
<th>Aspecific lesions</th>
<th>Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pill esophagitis</td>
<td>Drug treatment NSAIDs, antibiotics, AZT, potassium chloride, etc.)</td>
<td>Erosion, ulcer, acute inflammation, granulation tissue</td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td>Corrosive esophagitis</td>
<td>Ingestion of caustic substances (accidental, suicidal)</td>
<td>Diffuse necrosis</td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td>Radiation esophagitis</td>
<td>Clinical history of radiation therapy</td>
<td>Cell atypia, vascular changes</td>
<td>Ulcer, edema, fibrosis</td>
<td>HE</td>
</tr>
<tr>
<td>Chemotherapy esophagitis</td>
<td>Clinical history of chemotherapy</td>
<td>Focal inflammation, granulomas</td>
<td>Epithelial nuclear atypia, necrosis, granulation tissue</td>
<td>HE</td>
</tr>
<tr>
<td>Esophagitis in Crohn’s disease</td>
<td>Enteric symptoms, topography of lesion (focal ulcers)</td>
<td>Ulcer</td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td>Esophagitis in connective tissue disease</td>
<td>Clinical history</td>
<td>Microscopic esophagitis-like lesions</td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td>Skin diseases</td>
<td>Pemphigus vulgaris</td>
<td>Intraepithelial bullae, acantholysis, lymphocytes and eosinophils</td>
<td>IF (IgG and C3 deposits in intercellular spaces)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hailey-Hailey disease</td>
<td>Suprabasal bulla</td>
<td>HE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bullous pemphigoid</td>
<td>Subepithelial bulla, mild inflammation</td>
<td>IF (IgG deposits in basal membrane)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lichen planus</td>
<td>Vacuolization in the basal area, lymphocytic infiltration</td>
<td>HE</td>
<td></td>
</tr>
</tbody>
</table>

HE: hematoxylin-eosin; IF: immunofluorescence.

- Findings are “suggestive” of eosinophilic esophagitis (≥15 eosinophils/HPF, with unknown clinical context) or “diagnostic” for eosinophilic esophagitis (≥15 eosinophils/HPF in a coherent clinical context).
- Findings are “diagnostic” for specific esophagitis (Candida, CMV, Herpes).

4. Barrett’s esophagus

4.1. Definition

Barrett’s esophagus (BE) is defined as columnar metaplasia of the distal esophagus, is caused by chronic GERD and represents a risk factor for esophageal adenocarcinoma [27].

Two types of columnar epithelium may replace esophageal stratified squamous epithelium*** [10]:
1. intestinal-type epithelium
2. cardia-type epithelium.

This definition is the result of several reassessments of histologic and endoscopic criteria that have led to different definitions of BE over time [10,28,29]. Therefore, the adopted definition of BE is based on the current literature and reflects the viewpoint of the authors of this article; however, advances in our understanding of BE may be expected in the near future.

The original description also included a fundic type of Barrett’s esophagus [28]. However, when oxyntic mucosa is found in the distal esophagus, it is generally a sign of hiatus hernia, whereas it represents ectopia (the so-called “inlet patches”) when found in the mid-proximal esophagus.

4.2. The border area

Even though the “anatomical” gastro-esophageal junction (GEJ) has been variously defined, a recent consensus paper [30] identifies it with the “proximal border of the gastric folds” when endoscopy is performed with minimal air insufflation (Fig. 6A). The “histological” squamo-columnar junction (SCJ) or Z line is the transition between esophageal squamous epithelium and the glandular epithelium of the stomach (cardia mucosa): it can be detected endoscopically due to the colour change (white vs. pinkish). As a rule, the two junctions overlap, but SCJ may be located up to >1 cm proximally to GEJ also in normal subjects [31].

4.3. Endoscopic description

According to the Montreal classification [10], the term ESEM (Endoscopically Suspected Esophageal Metaplasia) designates the presence of “salmon pink” mucosa in the distal esophagus at endoscopy: it describes suspected columnar metaplasia (in the tubular esophagus), to be confirmed by histology***.
Endoscopic diagnosis is carried out in three steps [30]:
1. identification of the anatomical gastro-esophageal junction (GEJ);
2. detection of the squamo-columnar junction (SCJ or Z line);
3. measurement of columnar segment.

The “C&M” criteria of the Prague classification [30] provide clear rules for reporting endoscopic description and extent of ESEM.

ESEM is measured by two descriptors expressed in cm from GEJ (Fig. 6B):
- C: circumferential extent
- M: maximal extent

Endoscopic findings measured in this way can be classified into three categories:
- LSBE: Long Segment Barrett’s Esophagus, ≥ 3 cm;
- SSBE: Short Segment Barrett’s Esophagus, < 3 cm;
- irregular SCJ/Z line: irregular SCJ < 1–0.5 cm (which does not define ESEM with certainty).

The “C&M” criteria have proved to be highly reliable and reproducible (> 90%) both in GEJ detection and in defining SSBE and LSBE. On the other hand irregular Z line (or “ultra short” BE), deals with a heterogeneous group of endoscopic patterns including SSBE with M < 1 cm which is characterized by low reproducibility [30,32,33].

LSBE and SSBE share the same epidemiology and likely represent two phases of the same process [27]. The length of the metaplastic segment and the presence of intestinal metaplasia are currently considered major risk factors for adenocarcinoma [34,35].

4.4. Biopsy sampling

Multiple biopsies are required in order to characterize ESEM, their number being correlated to ESEM length [39]: 8 biopsies in the metaplastic segment allow detection of intestinal epithelium in 68% of cases [40], but if biopsies are performed close to squamo-columnar junction, the percentage rises to 94% even with fewer samples [41]. In patients being followed up for BE, biopsies are recommended in the 4 quadrants of the distal esophagus (every 2 cm routinely and every 1 cm in case of dysplasia [42,43]: unfortunately this sampling is not frequently performed in routine practice. The widespread availability of new advanced endoscopic techniques (narrow band imaging and magnification chromoendoscopy) is expected to increase the detection of dysplasia through a better targeting of biopsy sampling.

4.5. Individual histologic lesions

Cardia epithelium: columnar epithelium characterized by mucous-secreting cells and by PAS+ antral-like glands (Fig. 7). Superficial columnar cells store both neutral and acid mucins; therefore, the presence of variable alcianophilia

![Fig. 7. Cardia-type (or junctional) columnar metaplasia: antral-like glands and gastric foveolar epithelium in the absence of goblet cells. (H-E, 20×)](image)
in the absence of goblet cell morphology is not sufficient to diagnose intestinal metaplasia. Similarly, the presence of PAS+ dystrophic columnar cells (pseudo-goblet cells) does not entail intestinal metaplasia. Cardia glands may show pancreatic acinar metaplasia.

**Intestinal epithelium:** columnar epithelium characterized by goblet cells storing acid mucins. The most common variant of intestinal metaplasia is the “incomplete type” (Fig. 8A), i.e. devoid of absorptive and Paneth cells. Columnar cells intermingled with goblet cells often show mixed PAS and Alcian blue-positive mucins (Fig. 8B). The base of the crypts may be distorted and crowded, a finding that can be easily misinterpreted as “dysplasia” (Fig. 9).

**Oxyntic gastric epithelium:** surface mucous-secreting epithelium with underlying oxyntic glands. As previously reported, the presence of oxyntic mucosa does not lead to BE diagnosis.

### Problems in diagnostic assessment of Barrett’s esophagus:

BE uncertainty areas (due to the different definitions that have been adopted over the years) regard the “short” forms and/or the absence of intestinal metaplasia:

- **Intestinal metaplasia:** it is the main finding required for BE diagnosis in American and European literature [36], while British definition covers any “columnar” metaplasia, including cardia metaplasia [37].
- **Extent of the metaplastic segment:** the “C&M” criteria are highly reproducible for LSBE and SSBE (>1 cm). Conversely, the reproducibility of “ultra-short” BE or irregular Z line (irregularity <1 cm) is poor [30,33]. Intestinal metaplasia of the cardia (non-ESEM) is a frequent finding (15–20%) and seems not to be associated with significant cancer risk.
- **Cost/effectiveness:** the follow up of BE is expensive [37, 38]. A “broad” definition of BE (including ultra-short BE without intestinal metaplasia) leads to an increase in follow-up costs, and makes it ineffective [32].

### 4.6. Diagnostic procedure

**Algorithm: endoscopy + histology = diagnosis**

BE diagnosis is the result of a two-step procedure [10]:

1. **Endoscopy**
2. **Histology**

Endoscopic detection of ESEM must be confirmed by histology***.

ESEM becomes BE in the presence of intestinal epithelium, regardless of the endoscopic length of the metaplastic segment (≥1 cm).
ESEM becomes BE in the presence of cardia epithelium only in LSBE: in this case re-sampling close to the SCJ easily leads to the detection of intestinal epithelium [44].

The presence of intestinal epithelium without ESEM does not lead to BE diagnosis: the appropriate definition in this case would be “intestinal metaplasia of the cardia” [31].

“Irregular Z line” defines a doubtful condition that does not enable a diagnosis of BE due to the equivocal endoscopic picture, regardless of histological findings [32].

**Dysplasia (or non-invasive neoplasia) in BE** is defined by unequivocal neoplastic change that does not extend beyond the basal membrane. Dysplasia requires exclusion of regenerative lesions [45].

Dysplasia in BE does not equate with atypia and is not expected to regress. Dysplasia is both a precursor of adenocarcinoma and a cancer-associated lesion [46].

Dysplasia is endoscopically associated with flat, raised or depressed lesions and is defined by combined cytological atypia and architectural abnormalities (Table 5). The diagnostic criteria of dysplasia in BE are common to those used in other parts of the gastro-intestinal tract [45].

Dysplastic glands show architectural changes (gland fusion and budding, variable gland size, cribriform pattern) and cytological alterations (hyperchromatic and elongated nuclei, nuclear stratification) causing an increasing morphological deviation from the metaplastic phenotype. Cytological and architectural abnormalities must involve the entire length of glands (Fig. 10).

| Table 5 |
| Dysplasia/non-invasive neoplasia: criteria to be considered |
| 1. Cytological abnormalities |
| 2. Architectural abnormalities |
| 3. Epithelial maturation |
| 4. Presence of associated inflammation |

| Fig. 10. Low-grade epithelial dysplasia in Barrett’s esophagus: cytological atypia extends to the entire thickness of the epithelium, including the surface (top left). (H-E, 20×) |

**Caveat**
- dysplasia must extend to the surface epithelium;
- the presence of erosion and/or active inflammation requires caution in diagnosing dysplasia.

Dysplasia associated with BE is divided into three diagnostic categories [47]:
1. Low Grade Dysplasia = LGD
2. High Grade Dysplasia = HGD
3. Indefinite for dysplasia

**LGD** is characterized by scanty architectural distortion and mild cytological atypia, mainly affecting the lower half of the crypts. Hyperchromic nuclei with irregular contours, nuclear overlapping and dystrophic goblet cells may be present.

**HGD** is characterized by more complex glandular distortion; nuclear pleomorphism, hyperchromasia and nuclear stratification are more severe.

**Indefinite for dysplasia:** this term only applies to cases where the pathologist cannot decide with certainty whether the lesion is hyperplastic/regenerative or neoplastic in nature. This may be due to inadequate biopsy sampling (e.g. poorly oriented biopsies that do not enable full thickness assessment) or to the presence of cytological atypia and/or structural alterations of doubtful interpretation. This could also depend on the specific experience of the pathologist. It is a “provisional” diagnosis that must be followed by short-term resampling and/or second opinion. The presence of erosion and neutrophilic infiltrate requires additional caution in diagnosing dysplasia.

4.7. Special methods (Table 6)

**Alcian Blue-PAS:** it helps distinguish cardia from intestinal epithelium; the latter only shows goblet cells with Alcian blue-positive acid mucins.

**Cytokeratins:** cytokeratins 7 and 20 have been suggested as a tool to differentiate intestinal epithelium in BE from gastric intestinal metaplasia [48]. Far from providing conclusive results, the validation of this panel has often revealed false positives/negatives [49].

**CDX-2:** this homeobox gene promoting intestinal phenotype is useful to demonstrate the presence of intestinal differentiation [50]. However, intestinal phenotype is also detectable with hematoxilin-eosin, in combination with Alcian blue-PAS.

| Table 6 |
| Special techniques |
| 1. Alcian Blue-PAS: recommended |
| 2. CDX-2: not recommended |
| 3. CK7/CK20: not recommended |
| 4. MUC: not recommended |
| 5. p53: optional for dysplasia |
| 6. Mib-1/Ki-67: optional for dysplasia |
MUC 1-2-3-4-5: family of genes coding for the glycoproteins (mucins) that make up intra- and extracellular mucus. The distribution of MUC in the gastrointestinal tract varies (e.g. MUC 2 is more frequent in colonic epithelium). Cross-reactivity of commercial antibodies has been reported [51].

p53: nuclear accumulation of p53 can help differentiate between a reactive/regenerative lesion and dysplasia, with strong positivity favouring dysplasia. However, it should be taken into account that regenerative metaplastic lesions may be associated with a variable increase in p53 and, on the other hand, dysplasia and carcinoma do not always accumulate p53 [47].

Mib-1/Ki-67 shows the increased extent of proliferation in case of dysplasia. However, regenerative lesions may also be associated with high proliferation rate.

In conclusion, so far no specific and reliable markers can replace conventional histologic examination in the diagnosis of BE.

4.8. How to write a diagnosis

The flow chart below describes the way in which a diagnosis should be drafted, based on (1) histological findings, and (2) endoscopic pattern.

The histology report should include information regarding (1) the distance from incisors of GEJ and SCJ, (2) the length of metaplastic segment (according with the C&M criteria), and (3) the site of biopsies, as in the following example:

“GEJ at 40 cm; endoscopically suspected metaplastic segment C1-M3; 2 biopsies in the circumferential area (a) and 2 in a more proximal tongue close to SCJ (b).
(a) Two samples of cardia-type mucosa with focal intestinal metaplasia (incomplete type).
(b) Two samples of squamous epithelium with focal intestinal metaplasia (incomplete type).
Considering the endoscopic description, the above findings are diagnostic for Barrett’s esophagus with intestinal metaplasia.”

*The term BE is suggested in this case because it is very likely that a subsequent bioptic sampling, closer to the Z line (SCJ), will show the presence of intestinal metaplasia [44].
In the absence of a complete information about the endoscopic picture and biopptic sampling, the histological diagnosis should be merely “descriptive”.

5. Future perspectives

These “diagnostic guidelines” have been prepared with practical needs in mind and refer to the current literature according to which BE length and the presence of intestinal metaplasia are the main cancer risk factors. However, recent studies seem to demonstrate that:
• even cardia-type BE may have some degree of intestinal differentiation [52];
• both cardia and intestinal BE show similar alterations in DNA content [53];
• many esophageal “minute cancers” originate in a non-intestinal background [54].

The real impact of these observations on diagnostic practice (i.e. the reassessment of risk in cardial BE and of the management of such patients) is still to be confirmed [55].

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Conflict of interest

The authors have no conflicts of interest to disclose.

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